Claims:

1. A human influenza virus comprising an RNA-sequence encoding a modified RNA-polymerase which differs from the wild-type RNA-polymerase of said human influenza virus in that at least one of the amino acid residue(s) distinguishing the wild-type RNA-polymerase of said human influenza virus from FPV Bratislava RNA-polymerase has been replaced with the corresponding amino acid residue(s) as present in FPV Bratislava RNA-polymerase.

2. The influenza virus of claim 1, which is selected from influenza A including strains of type H1N1, H2N2 and H3N2, influenza B and influenza C, and preferably is an influenza A type H1N1, including WSN/33, PR8/34 or the like, an influenza A type H2N2, including Asia/57 or the like, or an influenza A type H3N2, including Victoria/68 or the like.

3. The influenza virus of claim 1 or 2, wherein the at least one distinguishing amino acid residue to be replaced is located within the PB1 segment of the virus.

4. The influenza virus of claim 3, wherein at least one or all of the following PB1 amino acid substitutions S384P, L396I, L628M, V644A, T741A, relative to the wild-type WSN-PB1 polypeptide shown in SEQ ID NO:25 have been effected, preferably the influenza virus strain used is WSN-K68 carrying five distinguishing amino acids.

5. The influenza virus of claim 3, which encodes the PB1 segment shown in SEQ ID NO:27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47, preferably said influenza virus comprises a PB1 (segment2) RNA sequence corresponding to the nucleotide sequence shown in SEQ ID NO:26, 28, 30, 32, 34, 36, 38, 40, 42, 44 or 46.

6. The influenza virus according to any one of claims 1 to 5, wherein the modified RNA-polymerase is capable of recognition of segments with modified vRNA promoter sequences resulting in an enhanced rate of transcription and/or replication relative to said wild-type human influenza virus RNA-polymerase.

7. The influenza virus of claim 6, wherein the segments with modified vRNA promoter sequences contain terminal viral RNA sequences which have been modified by nucleotide substitutions in up to five positions, resulting in improved transcription rates of both the vRNA promoter as well as the cRNA promoter as present in the complementary sequences.

8. The influenza virus of claim 7, wherein the 12 nucleotide conserved influenza 3' terminal sequence has been modified by replacement of one to three nucleotides occurring in said sequence at positions 3, 5 and 8 relative to the 3' end by other nucleotides, and/or wherein the 13 nucleotide conserved influenza 5' terminal sequence has been modified by replacement of one or two nucleotides occurring in said sequence at positions 3 and 8 by other nucleotides.

9. The influenza virus of claim 8, wherein the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A and C8U, or G3C and C8G, preferably the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A, U5C and C8U, or G3C, U5C and C8G.

10. The influenza virus of claim 9, which comprises a 3' terminal nucleotide sequence of (5')-CCUGUUUCUACU-3' or (5')-CCUGUUUUUACU-3'.

11. The influenza virus according to any one of claims 7 to 10, wherein the 5' terminal nucleotide sequence comprises the modifications U3A and A8U resulting in a 5'-terminal sequence of 5'-AGAAGAAUCAAGG.

12. The influenza virus according to any one of claims 1 to 11 which is suitable for high yielding expression of one or more foreign recombinant or altered viral proteins, preferably said influenza virus contains
(i) one or more segment(s) with a foreign recombinant or altered viral gene sequence in addition to the RNA segments of the normal viral genome (additional segment) or partially replacing them (replacing segment), whereby the additional segment(s) and replacing segment(s) comprise the foreign or altered gene encoding the protein to be expressed in monocistronic arrangement and have modified vRNA promoter sequences as defined in claims 7 to 11; and/or
(ii) one or more bicistronic vRNA segment(s), preferably in ambisense or in tandem arrangement, whereby the bicistronic vRNA segment(s) has/have foreign gene(s) encoding the protein(s) to be expressed and being in covalent linkage with one of the authentic viral genes, preferably the neuraminidase gene, and has/have modified vRNA promoter sequences as defined in claims 7 to 11.

13. The influenza viruses according to claim 12 having at least one additional segment coding for one or more foreign genes or one or more altered viral genes in monocistronic arrangement.

14. The influenza virus according to claim 13 in which the at least one additional segment codes for a glycoprotein of foreign-viral, animal, human or other origin, with or without in-frame fusion linkage to influenza coding sequences, in which the glycoprotein is at the same time incorporated itself in the virion envelopes, preferably the foreign glycoprotein sequence incorporated as vRNA and as a protein is derived from the genome of the contagious swine fever virus (CSFV), the bovine viral diarrhea virus (BVDV), the vesicular stomatitis virus (VSV), the Borna virus (BDV), the Marburg virus, the Ebola virus, the hepatitis C virus, the tick-borne meningo-encephalitis virus (TBE), the Western Nile virus or the human immunodeficiency virus (HIV).

15. The influenza virus according to claim 13 in which one or more of the incorporated foreign genes code

(a) for a lymphokine of human or animal origin which is secreted by the influenza vector infected cell, or

(b) for the expression of an apoptosis-inducing gene or a toxin gene, effective in the primarily infected cell or, after secretion, in neighboring cells.

16. The influenza virus of claim 12, which is genetically stable in the absence of any helper virus and which comprises at least one viral RNA segment being an ambisense RNA molecule (ambisense RNA segment) and containing one of the standard viral genes in sense orientation and a foreign, recombinant gene in anti-sense orientation, or *vice versa*, in overall convergent arrangement.

17. The influenza virus of claim 16, wherein at least one of the regular viral RNA segments is replaced by an ambisense RNA segment which contains one of the standard viral genes in sense orientation and a foreign, recombinant gene in anti-sense orientation, or *vice versa*, in overall convergent arrangement.

18. The influenza virus of claim 16 or 17, wherein in the ambisense RNA molecule said foreign recombinant gene is covalently bound to one of the viral genes, while the original vRNA segment coding for the same gene is deleted from the recombinant virus by way of specific ribozyme cleavage or is left out from the set of RNA-polymerase I promoted vRNA synthesizing plasmids, able to result in infectious viruses.

19. The influenza virus according to any one of claims 16 to 18, wherein one or more of the standard viral RNA segments, differing from said at least one ambisense RNA segment, comprises a vRNA encoding a foreign gene, preferably one or more of the regular viral RNA segments has (have) been exchanged for a vRNA encoding a foreign gene, preferably

one or both of the standard glycoproteins hemagglutinin and neuraminidase have been exchanged into foreign glycoprotein(s) or into fusion glycoproteins consisting of an anchor segment derived from hemagglutinin and an ectodomain obtained from the foreign source, viral or cellular, or in which such recombinant glycoprotein has been inserted as a third molecular species in addition to the remaining standard components.

20. The influenza virus of claim 12, which is genetically stable in the absence of any helper virus and which comprises at least one viral RNA segment being a bicistronic RNA molecule coding for two genes in tandem arrangement (tandem RNA segment), in said tandem RNA segment one of the standard viral genes being in covalent junction with a foreign, recombinant gene and said tandem RNA segment having an upstream splice donor and a downstream splice acceptor signal surrounding the proximal coding region.

21. The influenza virus of claim 20, wherein the tandem RNA segment contains one of the standard viral genes in distal mRNA position behind a foreign, recombinant gene in proximal position, or vice versa, both in antisense orientation with regard to the viral RNA as present within the virus.

22. The influenza virus of claim 20 or 21, wherein at least one of the regular viral RNA segments is replaced by a tandem RNA segment, preferably the replaced regular viral RNA segment is selected from the neuraminidase segment, hemagglutinin segment and NS segment.

23. The influenza virus according to any one of claims 20 to 22, wherein the splice donor and splice acceptor signals are selected from sequences as present in influenza WSN segment 7 and 8 or other partially effective splice reacting substrates, preferably the splice donor and splice acceptor

signals are selected from sequences as present in influenza WSN segment 7.

24. The influenza virus according to any one of claims 20 to 23, wherein one or more of the regular viral RNA segments, differing from said at least one tandem RNA segment, comprises a vRNA encoding a foreign gene which may or may not be in covalent connection to one of the viral genes, and preferably one or more of the regular viral RNA segments has (have) been deleted and replaced by a tandem vRNA encoding in addition a foreign gene.

25. The influenza virus according to any one of claims 20 to 24, in which the foreign gene(s) in the tandem RNA segment

(i) code for proteins and/or glycoproteins which are secreted from cells infected with the recombinant virus;

(ii) code for proteins or artificial polypeptides designed to support an efficient HLA-restricted presentation of inherent epitopes at the surface of infected cells, for stimulation of a B cell and/or T cell response;

(iii) is a nucleotide sequence causing viral attenuation, preferably the foreign gene is coding for part of the viral neuraminidase gene in inverted, i.e. sense orientation, with or without an inserted ribozyme sequence,

preferably the tandem segment part of the neuraminidase gene in sense orientation is attached to the hemagglutinin vRNA segment, and optionally another gene or reporter gene is encoded in a second tandem vRNA, preferably in conjunction with NS2.

26. The influenza virus according to any one of claims 16 to 25 which is suitable for the expression of non-influenza genes or synthetic genes, or gene-inhibitory sequences such as, but not limited to, antisense genes or ribozymes, whereby

(i) the non-influenza genes are covalently linked to one of the viral genes,

(ii) the non-influenza gene constitutes a membrane glycoprotein consisting of a fusion of the viral HA transmembrane and cytoplasmic regions with the foreign ectodomain sequence.

27. A non-avian, non-human influenza virus, preferably an equine or a porcine influenza virus comprising an RNA-sequence encoding a modified RNA-polymerase which differs from the wild-type RNA-polymerase of said non-avian, non-human influenza virus in that at least one of the amino acid residue(s) distinguishing the wild-type RNA-polymerase of said non-avian, non-human influenza virus from FPV Bratislava RNA-polymerase has been replaced with the corresponding amino acid residue(s) as present in FPV Bratislava RNA-polymerase, preferably said influenza virus is as defined in any one of claims 2 to 26.

28. A process for preparing the influenza virus of claims 1 to 27 which comprises replacing the RNA-sequence encoding the wild-type RNA-polymerase of said influenza virus with an RNA-sequence encoding the modified RNA-polymerase.

29. The process of claim 28, which is suitable for preparing PB1-chimeric viruses as defined in claims 1 to 11 and 27 as well as recombinant viruses as defined in claims 12 to 27, said viruses being generated via cotransfection of up to eight cDNA plasmids containing the viral cDNAs, or chimeric (segment 2: PB1) and bicistronic recombinant (segment 6: NA/foreign gene) cDNA sequences instead, in such a way that they are transcribed *in vivo* by both RNA-polymerase I and RNA-polymerase II and jointly give rise to progeny viruses according to the plasmid insert design.

30. A pharmaceutical composition comprising the influenza virus according to any one of claims 1 to 27.

31. The pharmaceutical composition of claim 30 which is suitable

(i) for gene transfer into cells, preferably into mammalian cells, more preferably into human cells, by recombinant viral infection;
(ii) for gene transfer into antigen-presenting cells, preferably into dendritic cells, and the use of the obtained product for *ex vivo* immunotherapy;
(iii) for *in vivo* somatic gene therapy;
(iv) for *in vivo* vaccination, including therapeutic and prophylactic vaccination;
(v) for eliciting an immune response, including the induction of a T-cell response;
(vi) for treating a growing tumor or a chronic infectious disease.

32. Use of the influenza virus according to any one of claims 1 to 27 for preparing an agent
(i) for gene transfer into cells, preferably into mammalian cells, more preferably into human cells, by viral infection;
(ii) for gene transfer into antigen-presenting cells and the use of the obtained product for *ex vivo* immunotherapy;
(iii) for *in vivo* somatic gene therapy;
(iv) for *in vivo* vaccination, including therapeutic and prophylactic vaccination;
(v) for eliciting an immune response, including the induction of a T-cell response;
(vi) for treating a growing tumor or a chronic infectious disease.

33. A method for
(i) gene transfer into cells, preferably into mammalian cells, more preferably into human cells, by viral infection;
(ii) gene transfer into antigen-presenting cells, and the use of the obtained product for *ex vivo* immunotherapy;
(iii) *in vivo* somatic gene therapy;
(iv) *in vivo* vaccination, including therapeutic and prophylactic vaccination;

(v) eliciting an immune response, including the induction of a T-cell response, preferably a CD4+ T-cell response, a CD8 T-cell response or both, or the induction of an antibody response;

(vi) treating a growing tumor or a chronic infectious disease;

(vii) preparing a vaccine;

(viii) preventing and/or treating influenza;

which comprises contacting the cells, the antigen-presenting cells, the person or the patient in need for vaccination, for influenza treatment or for somatic gene therapy, or cell cultures with the influenza virus according to any one of claims 1 to 27.

34. A method for the production of proteins or glycoproteins which comprises utilizing the influenza virus according to claims 1 to 27 as expression vector, preferably the production method is performed in cell culture cells or in fertilized chicken eggs.

35. Use of the influenza virus according to claims 1 to 27 for preparing agents

(i) for transfer and expression of foreign genes into cells infected by such viruses, or

(ii) for transfer and expression of RNA molecules into cells infected by such viruses, preferably the RNA molecules to be expressed are antisense sequences or double-strand sequences relative to the target cell cellular mRNA molecules, and/or the agent is suitable for sequence-specific gene silencing, preferably by antisense RNA or RNA interference mechanisms such as ribozyme cleavages of target RNAs.

36. A method for transfer and expression of foreign genes into cells, and for transfer and expression of RNA molecules into cells, which method comprises infecting the cells with the influenza virus according to claims 1 to 27.

37. Use of the influenza virus according to claims 1 to 27 for preparing agents for immunotherapy, preferably for autologous immunotherapy.

38. A method for an immunotherapy which comprises *ex vivo* infection of immune cells, preferably dendritic cells, with the influenza virus according to claims 1 to 27, and introduction of the transduced cells into the patient.

39. A method to elicit an immune response directed against an antigen, comprising the steps of introducing the influenza virus as defined in claims 1 to 27, preferably the human influenza virus as defined in claims 1 to 26, into a cell or administering it to a mammal, wherein said influenza virus contains at least one foreign gene encoding the antigen.

40. The method of claim 39, wherein said foreign gene encoding the antigen is a polynucleotide sequence associated with a disease, preferably an infectious diseases, or a tumor disease, preferably the antigen is exemplified by, but not limited to,

(i) virus-associated antigens such as the HIV antigens gp160, gp 120, rev, tat, NC, the HBV e-antigen or core antigen, the HPV E6 or E7 antigen, the herpes simplex virus glycoproteins or core proteins, other herpesvirus antigens and further viral and microbial antigens known to those skilled in the art, or

(ii) tumor associated antigens, especially the so-called cancer testis-antigens exemplified by the MAGE, BAGE and GAGE family of antigens, the NY-ESO-1 antigen, the SSX antigens, exemplified by the HOM-MEL-40.

41. The method of claim 39 or 40, wherein the polynucleotide sequence

(i) is derivable from a cDNA library isolated from tumor cells, or testis cells, or virus-infected cells, or micriobially infected cells, or cell-lines,

(ii) is a fusion protein consisting of epitopes derived from one or more T-cell specific epitope sequences as present in viral or other pathogens, or in tumor associated antigens.

42. A vaccine for therapeutic or prophylactic purposes which is
(a) a human influenza virus vaccine comprising a human influenza virus as defined in claims 1 to 26 or in claims 39 to 41, preferably said human influenza virus encodes the antigen for a membrane protein and in addition contains the membrane protein in the viral envelope; or
(b) a non-human influenza virus vaccine, preferably an equine or porcine influenza virus vaccine, comprising a virus as defined in claim 27.

43. The vaccine according to claim 42, wherein the virus
(i) is capable of being attenuated according to the tandem attenuation mechanism;
(ii) is only capable of limited replication; or
(iii) is an inactivated virus.

44. Transduced cells, preferably antigen-presenting cells, obtainable by the method of claim 33, option (i) or (ii).

45. A vaccine comprising transduced cells as defined in claim 44, preferably comprising transduced antigen-presenting cells, more preferably transduced dendritic cells, and most preferably mature dendritic cells, wherein said antigen-presenting cells are transduced *in vitro*.

46. A method to identify a polynucleotide sequence encoding at least one HLA-restricted epitope comprising the steps of

(a) preparing a gene bank or a cDNA bank from the cell or the microorganism to be tested;

(b) incorporating the cDNA or the DNA of the gene bank into the genome of the influenza virus as defined in claims 1 to 27 to yield recombinant virus particles,

(c) infecting immortalized autologous cells, which are capable of expression of HLA-class I molecules and/or HLA-class II molecules on their surface, with the recombinant virus particles obtained in step (b),

(d) expressing the proteins encoded by said cDNA or said DNA of the gene bank in the autologous cells and presenting the fragments of the proteins produced by the autologous cells or the cell surface in connection with HLA molecules;

(e) co-cultivating T-cells with the autologous cells; and

(f) stimulating the T-cells by such autologous cells which present antigens on their surface, whereby said antigens are recognized by the T-cells.

47. A method to study gene function in antigen presenting cells comprising steps (a) to (f) of claim 46.